

## A SHADOW RESPONSE IN A LARVAL CRUSTACEAN

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Most benthic crustaceans have planktonic larvae which, when responding to a light stimulus, show directional swimming (phototaxis). The sign of phototaxis usually varies depending upon light intensity. In general, the pattern consists of a negative response to high intensities and a positive response to low intensities (Thorson, 1964; Forward, 1976). Recent studies, however, indicate that at high intensities the sign of phototaxis for larvae of *Rhithropanopeus harrisi* (Forward and Costlow, 1974) and *Panulirus longipes cypnus* (Ritz, 1972) does not reverse from positive to negative, but overall responsiveness is reduced. In addition, as first shown by Herrnkind's (1968) work with *Uca pugilator* larvae and more extensively with light-adapted *R. harrisi* larvae (Forward, 1974a; Forward and Costlow, 1974), responses to high and low intensities by these species are the reverse of the normal pattern. Positive phototaxis occurs at high intensities, while a negative response is seen at low intensities.

Forward (1974a) suggested that for *R. harrisi* larvae the negative phototaxis, observed upon a sudden decrease in light intensity, may function for predator avoidance. Comparable behavior is observed in sedentary animals and is defined as a *shadow reflex*, i.e., a rapid withdrawal of exposed body parts in reaction to a shadow (Steven, 1963). Such a definition, however, is difficult to apply to a planktonic crustacean larva, which can respond with directionally-oriented movement upon an intensity decrease. *Shadow response* is perhaps a better term for such evasive movements and will be used throughout this report.

The present study is a continuation of earlier work (Forward, 1974a) providing further analysis of directional movements by *R. harrisi* larvae upon a sudden decrease in light intensity. An additional behavioral response considered herein is movement in relation to gravity. Larval crustaceans generally display a negative geotaxis in the absence of light and thereby remain swimming in the water column (Foxon, 1934; Sulkin, 1973). The sign of geotaxis can reverse to positive with changes in pressure (e.g., Rice, 1964), temperature (Ott and Forward, 1976; Parker, 1902), and salinity (Hughes and Richard, 1973). An apparent positive geotaxis can result from either active downward swimming or passive sinking. Since a taxis implies a locomotor response (Fraenkel and Gunn, 1961), passive sinking in response to a stimulus cannot be considered a true positive geotaxis. Perhaps it could be more accurately described as a *sinking response*.

Thus the present study proposes the existence of a shadow response in which avoidance behavior involves oriented responses to light and gravity. It is suggested that these behaviors would be appropriate for avoiding free swimming predators on zooplankton such as ctenophores.

## MATERIALS AND METHODS

Ovigerous specimens of *Rhithropanopeus harrisii* (Gould) were collected from the Neuse River in eastern North Carolina. All experiments were conducted with Stage I zoeae reared on a 12L:12D cycle at 25° C in filtered sea water at 25‰ salinity. Larvae were transferred daily to fresh sea water and were fed newly hatched *Artemia salina* nauplii. To avoid complications due to possible biological rhythms in behavior and changes during development, all experiments were begun four to six hours after the beginning of the light period on the second day after hatching. Larvae from at least three separate females were used for each experiment. The sample size reported for each experimental condition represents approximately equal numbers of larvae from each female.

Behavioral responses were monitored by placing 30–50 larvae in a lucite cuvette with a quartz entrance window, viewed horizontally by a stereomicroscope coupled to a closed circuit television system (described in detail in Forward, 1974b). The dark field microscope illumination system was interference-filtered to 802 nm (Optics Technology, Inc.; half band pass, 39 nm), a wave-length which neither alters nor induces photoresponses. The stimulus light source was a 150 w xenon arc lamp directed into a monochromator (Farrand Model F/3.5) set at 500 nm (full band pass 10 or 20 nm). Spectral purity was further regulated by a Corning No. 4-96 filter. This stimulus wavelength was chosen because a previous study of the spectral sensitivity indicated that 500 nm was the primary maximum (Forward and Costlow, 1974). Light intensity was regulated by neutral density filters and measured with a radiometer (YSI model 65). Stimulus duration was controlled by an electromagnetic shutter (Uniblitz model 225XOROX5, controlled by a model 310 drive unit). Unless otherwise stated the stimulus light was directed horizontally into the test cuvette. In some experiments, however, it was directed vertically from above. This was accomplished by interposing two prisms in the light path to elevate the beam, and then a front surface mirror was used to reflect the light down into the top of the test cuvette. To avoid problems due to the meniscus, the cuvette was carefully filled to a level parallel with its top.

Behavioral responses were recorded on video tape and analyzed as reported previously (Forward and Costlow, 1974). *Positive phototaxis* is defined as movement toward the stimulus source ( $\pm 15^\circ$ ), while *negative phototaxis* is movement directly away ( $\pm 15^\circ$ ). An additional behavioral response consisted of the cessation of swimming followed by downward sinking. This directly downward movement ( $\pm 15^\circ$ ) is defined as a *sinking response*.

The speed of movement during the sinking response was determined from measurements of the distance moved in the first 0.5 second after the termination of stimulation. True sinking speeds were also measured with larvae which were anesthetized by floating a drop of propylene phenoxylol on the surface of a well slide containing the larvae. Activity usually ceased within 20 minutes, after which larvae were transferred to fresh sea water and then gently pipetted into the top of the test cuvette positioned on the microscope stage. Sinking was recorded on video tape and speeds were determined by measuring the distances moved over a 0.5 second interval. The seawater salinity for these determinations was measured with a refractometer (American Optical Company—accuracy 1.0‰) and temperature in the cuvette was monitored with a temperature probe (YSI model 420)

coupled to a telethermometer (YSI model 44TD). Although the larvae were reared at 25° C, the room in which experiments were conducted was maintained at about 21° C; hence all sinking rates were performed at about this temperature. During the sinking determinations, larvae were narcotized but alive, since microscope examination indicated their hearts were beating. Greater than 95% of the animals always regained normal activity within one hour after transfer to fresh sea water. Mean speeds of movement were compared in a Student's *t*-test and significant differences tested at the five per cent level.

Conceptually, the experiments are based upon the previous finding (Forward, 1974a) that light-adapted *R. harrisii* larvae show a positive phototaxis to high intensity light and a negative response to lower intensities (Fig. 1A). Forward (1974a) suggested that these responses could participate in a shadow response during which swimming away from a potential predator occurs when its shadow falls upon a larval crustacean. The success of this behavior in avoiding predators is limited, however, because this response is only initiated upon exposure to an absolute low light intensity level, not upon a per cent decrease in intensity.

If a larva is being illuminated with light of an intensity comparable to that which occurs during the day and a shadow falls upon the animal, the light intensity could potentially decrease to either of three levels: (I) total darkness, (II) a low intensity at which negative phototaxis occurs, or (III) a higher intensity at which positive phototaxis would occur. A shadow response that functions under each of these three conditions would be most effective in predator avoidance. Thus the behavioral responses that result under these three stimulus conditions were investigated. In the experiments, the intensity of the entire stimulus beam is changed to mimic a shadow, rather than a portion of the pattern. The underlying assumption for this technique is that potential predators are much larger than an individual larva, and thus would cast a shadow over the entire animal.

The general procedure for any experiment was to light-adapt larvae under the room lights as well as a 60 w incandescent lamp for at least one hour prior to testing. Larvae were then transferred to the test cuvette which was positioned on the microscope stage. After pausing one minute in total darkness, light stimulation began. Larvae were then returned to the culture bowl and a new sample tested. Since the specific procedures varied with each experiment, these are described in detail under the Results section.

Another important consideration concerns identification of potential predators which a larva might avoid by using a shadow response. Postlarval fish occur locally in the Newport River Estuary during the summer months (Thayer, Hoss, Kjelson, Hettler and Lacroix, 1974), but gut contents indicate that they rarely feed on crab zoeae. This probably results because most zoeae are larger than the size range of organisms fed upon by postlarval fish (Kjelson, Peters, Thayer, and Johnson, 1975). Nevertheless, some small adult fish and ctenophores are reported to feed on meroplankton (Foxon, 1934). Thus feeding experiments were conducted with the small fish *Fundulus heteroclitus* and with the ctenophore *Mnemiopsis leidyi*. The latter is the most abundant ctenophore species in the Beaufort, North Carolina area and is found in great numbers during the summer months during which *R. harrisii* breeds (Schwartz and Chestnut, 1974).

The apparent optical density (O.D.) of the ctenophores along their oral-aboral

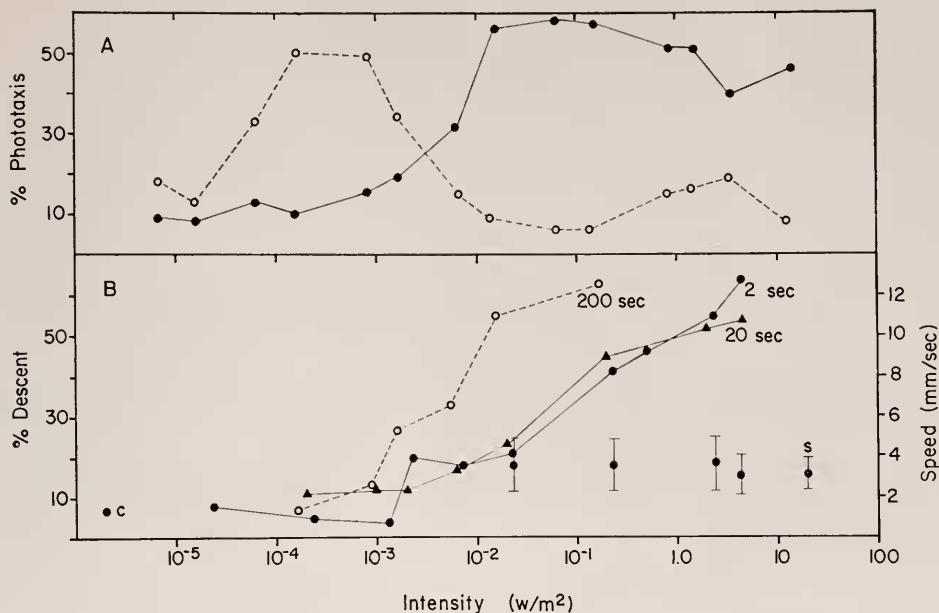


FIGURE 1. A. Per cent response (left ordinate) of positive (closed circle-solid line) and negative (open circle-dashed line) phototaxis to various stimulation intensities of 500 nm light (10 nm full band pass) (abscissa) by light adapted Stage I zoeae (replotted from Forward, 1974a). B. Percentage of light-adapted larvae showing a descent (left ordinate) upon termination of 500 nm light stimulus at different intensities (abscissa) and duration of 2 (closed circles), 20 (closed triangles) and 200 (open circles) seconds. Average sample sizes for each intensity at the different times are 56, 68, and 44, respectively. Random movement in the vertically downward direction (C-closed circle) was determined with no stimulus present. Speeds of movement (right ordinate) were recorded during the descent (closed circles) after a 2 second stimulus at the various intensities and during sinking by anesthetized animals (S). Mean speeds were plotted and vertical lines indicate the standard deviation. The average sample size for the descent and sinking speeds are 29 and 150, respectively.

and transverse axes was determined by placing freshly collected individual animals in a glass cuvette (ID  $32 \times 55 \times 84$  mm), filled with clear water of the same salinity as that in which the animals were collected. The cuvette was positioned in the sample compartment of a Cary Model 11 spectrophotometer and continuously scanned from 650 to 350 nm. The direction of the scans for all animals was descending. Plots of the apparent O.D. were made at 10 nm intervals even though the data were recorded continuously. These plots are the average of five animals within three specified size ranges, generally representing small, medium and large centropores as found in the Beaufort, North Carolina area.

## RESULTS

### *Light intensity decrease from high intensity to total darkness*

The general behavioral response upon extinguishing the light is a descent, during which larvae move vertically downward. This response is not observed upon turn-



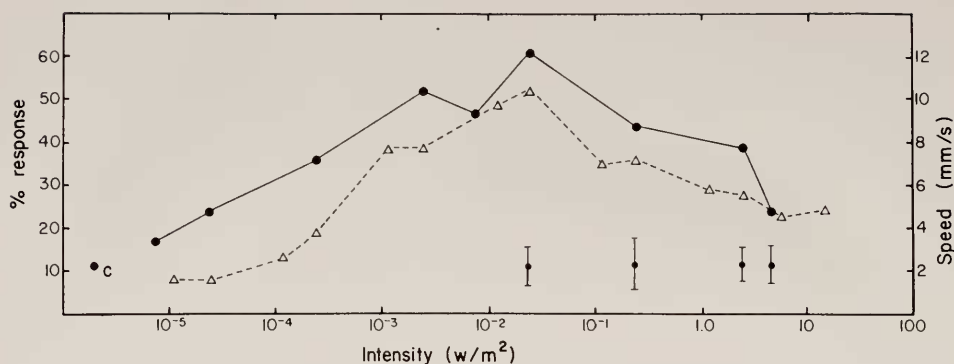


FIGURE 2. Per cent positive phototaxis (left ordinate) by dark-adapted larvae (open triangle-dashed line) upon stimulation with 500 nm light (10 nm full band pass) at different intensities (abscissa) (replotted from Forward, 1974a). The closed circles-solid line indicates per cent descent subsequent to termination of stimulation. The average sample size for each phototaxis and geotaxis point are 73 and 53, respectively. C-closed circle is the control level of descent (sample size is 73). The closed circles indicate the mean descent rates (right ordinate) at the different light intensities, and the vertical lines are the standard deviation. The average sample size is 19.

ing the light on. To establish the relationship between light intensity and this response, light-adapted larvae were stimulated at different intensity levels for 2, 20 and 200 seconds and upon extinguishing the light, the percentages of larvae showing the descent were determined. The procedure was to place larvae within the test cuvette upon the microscope stage for one minute (filtered microscope illumination only) and then stimulate three times for two seconds at 15 second intervals or two times with 30 seconds between termination and onset of stimuli for the 20- and 200-second duration stimuli. Each stimulus exposure for each larval preparation was at a different intensity. As seen in Figure 1B, the longer the stimulus duration, the greater the percentage of descending larvae at lower intensities. However, considering those intensities at which positive and negative phototaxis occur (Fig. 1A), it is apparent that the descent is only seen subsequent to stimulation at light intensities that initiate positive phototaxis.

This finding is further established by measuring per cent descent subsequent to stimulating larvae which were dark-adapted for two hours prior to testing. The technique was to pipette larvae into the cuvette under dim red 650 nm light. The preparation was placed on the microscope stage (802 nm illumination only) for one minute and then stimulated three times for two seconds each at different light intensities at 15 second intervals. Previous work (Forward, 1974a) indicated that after dark adaptation, negative phototaxis to low light intensities no longer occurred, and that the threshold intensity for positive phototaxis was lowered. In contrast, Figure 2 shows that the descent occurs in dark-adapted larvae and is greatest at those intensities which initiate the strongest positive phototactic responses.

The descent could result from either active swimming or passive sinking. To establish which of these alternatives was responsible, speeds of movement were determined during the descent subsequent to the 2-second stimulus at different intensities and for light-adapted anesthetized larvae under similar temperature

(average 21.3° C) and salinity (average 25.2‰) conditions. For both light- (Fig. 1B) and dark-adapted (Fig. 2) larvae, mean descent rates are not significantly different subsequent to different stimulus intensities. Furthermore, sinking speeds for light-adapted larvae are not significantly different from those during the descent subsequent to stimulation (Fig. 1B).

Therefore, three pieces of evidence indicate that the descent results from passive sinking. The observations of the anesthetized larvae show that if they stopped swimming, they would sink. In addition, the speeds during the descent are independent of stimulus intensity even though the per cent of larvae showing the response does change (Figs. 1B and 2). Finally, light-adapted anesthetized larvae sink at rates identical to those observed during the descent (Fig. 1B). Thus the descent observed upon extinguishing the light can be termed a sinking response.

Subjective determinations were made of the minimum time duration of a light intensity decrease necessary to induce the sinking response. Larvae were stimulated vertically for one minute at an average intensity of  $1.19 \text{ Wm}^{-2}$  (500 nm–20 nm full band pass). Then, at 10-second intervals, the light was extinguished for times ranging from 10 to 90 milliseconds (as timed by the shutter control unit—accuracy 5%). The slow speed control of the video tape unit made analysis of the direction of movements upon extinguishing the light for these times impossible, so the presence or absence of a response was made subjectively. Based on eight determinations, the minimum time length that the light must be off before a positive geotaxis was always observed was 30 milliseconds or longer, while 62% of the trials showed a response at 20 milliseconds, and no responses were seen at 10 milliseconds.

*Light intensity decrease from high intensity to that at which negative phototaxis occurs*

Previous work demonstrates that if larvae are stimulated with high intensity light, a positive phototaxis occurs. This can be reversed to negative, if the intensity is rapidly lowered to the range of about  $2 \times 10^{-3}$  to  $6.0 \times 10^{-5} \text{ W/m}^2$  at 500 nm (Forward, 1974a). While the results from these past experiments are interesting, the procedure was somewhat contrived, since the larvae were irradiated at high intensities for only a short amount of time before the light intensity was lowered. The more realistic sequence of a longer exposure followed by a decrease in intensity initiates a sinking response followed by the negative phototaxis.

To further investigate aspects of these responses, light-adapted larvae were irradiated for different lengths of time at two intensities that induce positive phototaxis. Then the intensity was lowered to a level that should induce negative phototaxis. The time delay between the onset of the sinking response and the beginning of negative phototaxis was determined from the recorded video tapes by continuously monitoring larval position and using the video tape frame number to indicate the time. The video tape deck records at 60 frames/second. Each larval preparation was tested under only one set of stimulus conditions.

Less than 15% of the larvae began negative phototaxis within 0.33 second after the onset of the light decrease under both stimulus conditions. For the remaining larvae the delay time was greater upon longer stimulation. A direct relationship exists between the delay time plotted on a logarithmic scale and the tested stimulus times for each of the two test intensities (Fig. 3). The least squares

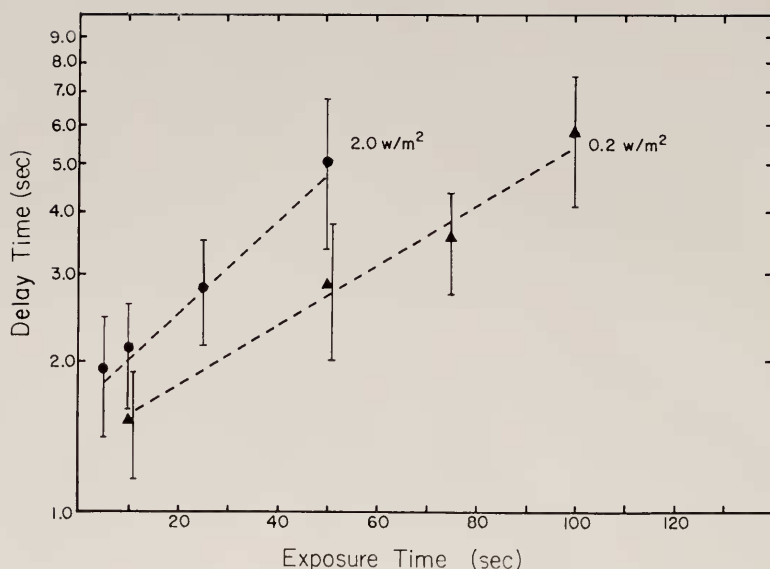


FIGURE 3. The time delay (ordinate) between the sinking response and negative phototaxis upon stimulation at 2.0 (solid circles) and 0.2 (solid triangles)  $\text{W/m}^2$  500 nm light (10 nm full band pass) for various times (abscissa) and decrease in intensity to  $2.0 \times 10^{-3} \text{ W/m}^2$ . The average sample size at each time and intensity and for the determination beginning at 2.0  $\text{W/m}^2$  was 31 and at 0.2  $\text{W/m}^2$  was 21.

regression lines were calculated from the original numbers even though the mean and standard deviation are plotted in Figure 3. The slopes of the lines are highly significant ( $P < 0.01$ ). Reciprocity is not seen for the delay time, since 10 seconds at 2.0  $\text{W/m}^2$  does not produce a similar delay time as 100 seconds at 0.2  $\text{W/m}^2$ .

*Light intensity decrease from high intensity to levels above those which induce negative phototaxis*

If larvae are stimulated at an intensity level that induces normal positive phototaxis and the light intensity is decreased within the range that should still produce positive phototaxis, a sinking response occurs. To determine the magnitude of the intensity change necessary to produce this response and also response dependence upon irradiation time length and intensity, the following experiment was performed. Separate groups of light-adapted larvae were irradiated under three separate conditions: 10 seconds at each of two intensities of 500 nm light (20 nm full band pass), which differed by a log unit and at the lower intensity for 50 seconds. At the end of these time periods, the intensity was decreased to a specific level by adding neutral density filters (represented as delta optical density), and the percentage of larvae showing the sinking response was determined. For the 10-second exposures each larval preparation received three sets of stimuli at 30-second intervals, while for the 50-second exposures, each preparation received only two sets of stimuli.

Upon stimulation in the horizontal direction (Fig. 4A), the optical density (O.D.) that must be added to induce the first clear sinking response under the three

stimulus conditions is independent of stimulus conditions as it ranges around 0.5. Similarly, under all three stimulus conditions an O.D. of 1.0 to 1.1 is needed to induce a maximal response. Increases in O.D. beyond this point do not greatly increase the response percentage.

To demonstrate that the observed sinking responses were not dependent upon stimulation from a horizontal direction, the same experiment was conducted with larvae stimulated vertically from above. These larvae were reared at 20‰, rather than 25‰, but previous unpublished data indicates that upon horizontal stimulation larval responses are identical. The results (Fig. 4B) are similar to those upon horizontal stimulation (Fig. 4A). The first clear sinking response occurs at 0.5 O.D. and the maximal responses occur at 1.0 to 1.1 O.D. and greater.

### Potential predators

Small fish and ctenophores are potential pelagic predators upon larval crustaceans. *Fundulus heteroclitus* was used as the representative of small fish. In the laboratory, it readily feeds upon stage I zoeae. In subjective experiments under different directional lighting conditions in both still water and with flowing currents of different speeds, it was concluded that larvae could not escape from a "hungry" *Fundulus*. So the usefulness of the shadow reflex is questionable in avoiding large predators which visually sight and actively pursue their prey.

In the Beaufort, North Carolina, area a major predator on small zooplankton is the ctenophore *Mnemiopsis leidyi*. Although the sea nettle *Chrysaora quinquecirrha* also occurs and feeds on zooplankton, it is usually less abundant (Schwartz and Chestnut, 1972). Under laboratory conditions, if larvae are introduced into an aquarium containing *M. leidyi* (under room lights), they are readily ingested by the ctenophores and are visible in the digestive system within 30 minutes.

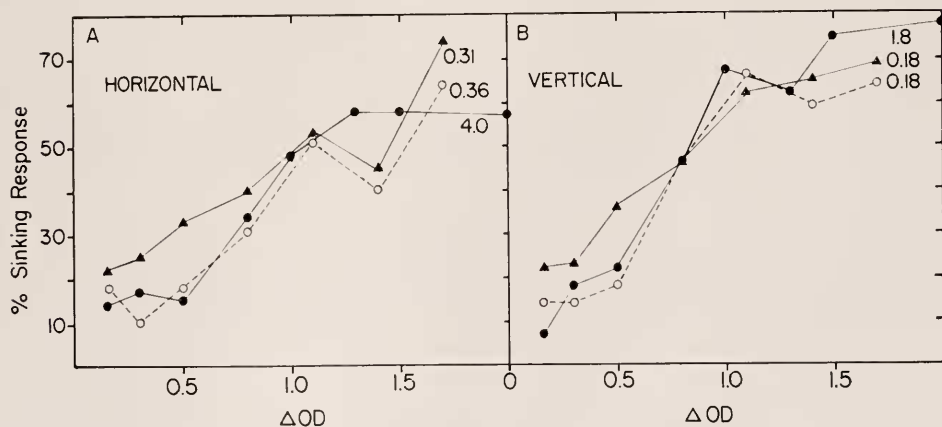


FIGURE 4. Per cent sinking response (ordinate) upon a decrease in light intensity by interposing neutral density filters in the stimulus beam path represented at delta O.D. (abscissa). Stimulation was from the horizontal (A) and the vertical (B) direction. The initial intensities (W/m<sup>2</sup>) are shown next to the appropriate curves, and the stimulus duration for the closed and open circle curves is 10 seconds, while that for the closed triangles is 50 seconds. The average sample size for each point in A and B are 67 and 84, respectively.



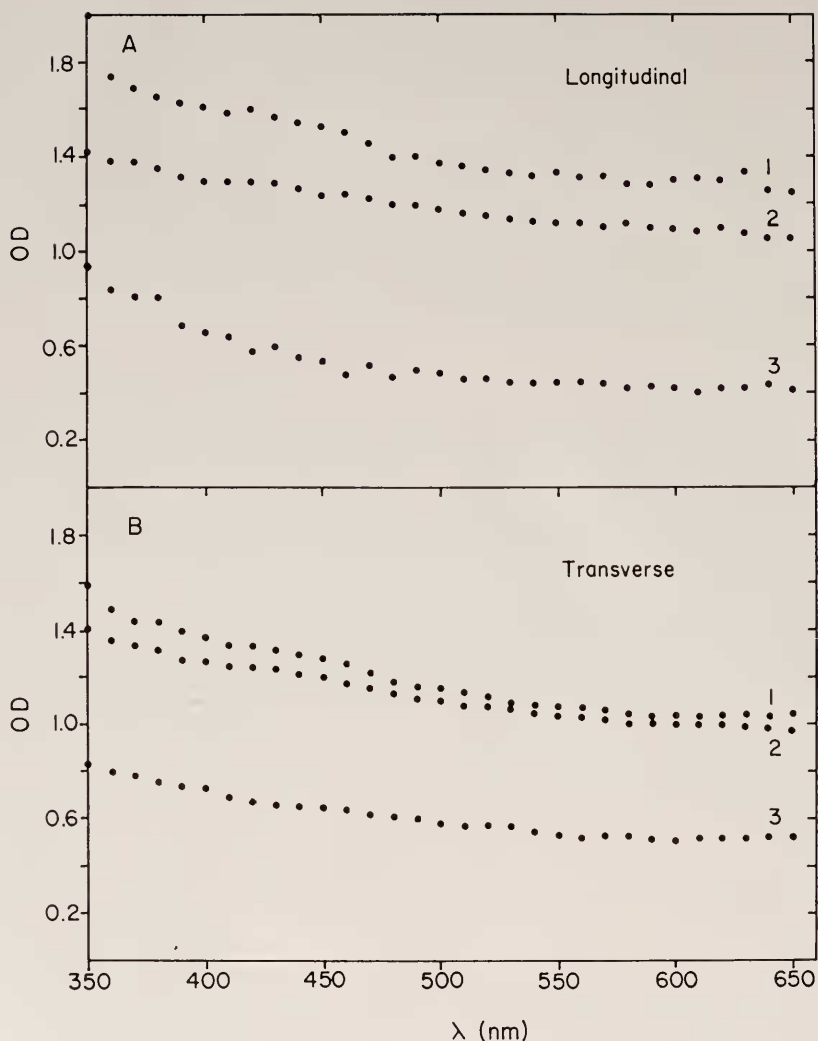


FIGURE 5. The apparent optical density (ordinate) upon longitudinal (A) and transverse (B) spectrophotometer scans from 650 to 350 nm (abscissa). The average dimensions of the three size ranges of *Mnemiopsis leidyi* tested were aboral to mouth distance 4.2 cm (1), 3.4 cm (2), 2.1 cm (3) and transverse or width distance 3.2 cm (1), 3.0 cm (2) and 1.9 cm (3). Points in each curve are averages from five animals.

Since *M. leidyi* appears to be almost transparent, a central question is whether this animal can attenuate light sufficiently to initiate the shadow response. Thus using a spectrophotometer, the apparent optical density (O.D.) due to both absorption and scattering along the transverse and longitudinal axes was measured. Since much of the attenuation results from scattering, the measured apparent O.D. may vary, depending upon the spatial relationship between the animals and the spectrophotometer light measuring system. The distance between the light exit slit and

entrance into test cuvette for all measurements was 2.5 cm. Thus the measured O.D. can be considered an approximation of the attenuation by the animals.

Figure 5 shows the O.D. of whole animals in three size ranges, which arbitrarily represent small, medium, and large animals as seen in the Beaufort, North Carolina, area. Generally, the O.D. gradually increases toward shorter wavelengths with no pronounced maxima. At 500 nm both medium and large specimens of *M. leidyi* have longitudinal and transverse O.D.s greater than 1.0 and thus could initiate a maximal sinking response. Animals in the smallest size range, however, have an O.D. around 0.5, which is at the threshold level for initiating the response.

To demonstrate that a sinking response could in fact occur upon encountering a ctenophore, light-adapted larvae were stimulated vertically with 500 nm light (20 nm full band pass; average intensity,  $0.73 \text{ W/m}^2$ ). During its horizontal optical path, the light passed through a rectangular leucite trough containing one *M. leidyi*. After a one-minute stimulation, the trough was pushed horizontally until the light passed through either the longitudinal or transverse body axis of the ctenophore, and the subsequent per cent sinking response was determined. Tests were run with eight *M. leidyi*, averaging 3.0 cm in width and 3.9 cm in length. According to Figure 5, these animals should have a transverse and longitudinal O.D. of about 1.1 and 1.3, respectively. The percentage of larvae moving vertically downward upon beginning the trough push was 14% ( $n = 409$ ). The per cent sinking response upon interposing the longitudinal axis of the ctenophores was 68% ( $n = 224$ ) and upon interposing the horizontal axis it was 57% ( $n = 272$ ). These per cent responses agree with values plotted in Figure 4B, which shows per cent responses of about 60% for these O.D.s.

#### DISCUSSION

Upon a light intensity decrease, stage I zoeae of the crab *Rhithropanopeus harrisii* show a shadow response consisting of directionally-oriented movements. Like the shadow reflex of barnacles (von Buddenbrock, 1930), the larval response only occurs upon an intensity decrease and not upon a change from total darkness to light. Although the exact behavioral responses and physiology depend upon the magnitude of the light intensity decrease, the larvae do show oriented movements under the three conditions originally proposed as necessary for a functional shadow response, *i.e.*, light intensity decrease from a high level (I) to total darkness, (II) to an intensity at which negative phototaxis occurs and (III) to a higher intensity at which positive phototaxis normally occurs.

If the initial intensity is sufficient to induce positive phototaxis and is suddenly decreased to darkness, a descent is observed for both light- and dark-adapted larvae. Comparison of the speeds of movement during the descent to those by anesthetized larvae indicates that the descent consists of passive sinking and as such is most accurately described as a sinking response. The sinking speeds by *R. harrisii* larvae are similar to those found for related species (Sulkin, 1973).

The minimum length of time that the light must be extinguished before the response is observed is 20 to 30 milliseconds. This indicates that the larvae are more sensitive to shadows than at least one barnacle species. Gwilliam (1963), in monitoring the shadow reflex of the barnacle *Mitella polymerus* by recording from the stalk nerve, found that shadows of less than 100 milliseconds in duration were not

perceived by the animal. Furthermore, since the shadow response occurs in both light- and dark-adapted larvae, the response presumably can function during both day and night.

If the light intensity is suddenly reduced to a level that can induce a negative phototaxis in light-adapted larvae (Fig. 1A), most larvae show a sinking response followed by the negative phototaxis. The length of time that the sinking response continues until the negative phototaxis begins is equal to the time delay between the two responses and lengthens with increasing intensity and duration of the initial light level. As previously reported (Forward, 1974a), the negative phototaxis is not initiated by a per cent change in intensity; rather the intensity must be reduced to an absolute level. The negative phototaxis would certainly be more effective than the sinking response in avoidance, since it involves directional movement away from an area of decreased light intensity (*i.e.*, the shadow) at swimming speeds which are faster (*e.g.*, mean speed at  $1.4 \times 10^{-3}$  W/m<sup>2</sup> is 8.6 mm/sec) (Forward, 1974a) than those at which the animals sink (mean is 3.1 mm/sec). However, the significance of this absolute light level, as related to naturally occurring light intensities, remains to be determined. An alternate explanation is that negative phototaxis participates in diurnal vertical migration. Since the negative response only occurs in light-adapted zoeae, a descent at the end of the day could result from negative phototaxis to low-light intensity levels (Forward, 1974a).

At higher light intensities the maximum sinking response is initiated upon an intensity decrease equivalent to reducing the light by 1.0 to 1.1 O.D. units or greater. This amount is independent of initial light intensity, duration and direction, since identical results occur upon vertical and horizontal stimulation. This value is also higher than that found neurophysiologically by Gwilliam (1963) for the barnacle *Mitella*, for which the maximum response occurred at intensity decreases equivalent to 0.4 O.D. units and greater. The lower threshold value for initiation of the sinking response is equivalent to a reduction of about 0.5 O.D. units. This value is considerably higher than that reported from behavioral studies of shadow reflexes in adult barnacles, in which decreases equivalent to reducing the light by 0.05 to 0.12 O.D. units are needed to initiate the reflex (Forbes, Seward and Crisp, 1971). Furthermore, crustaceans are capable of perceiving much lower light intensity changes, *e.g.*, the Weber fraction for adult *Daphnia magna* at white light intensities above 40 ergs/cm<sup>2</sup>/sec is equivalent to a 0.013 O.D. change (Ringelberg, Kasteel and Servaas, 1967).

Observations of fish feeding upon stage I zoeae suggest that larvae cannot escape large predators which visually sight and actively pursue their prey. The shadow response may be more effective in avoiding a passive predator such as the ctenophore *Mnemiopsis leidyi* which, during the summer breeding season for *R. harrisii*, is an abundant pelagic predator upon local zooplankton (Schwartz and Chestnut, 1974). Like most ctenophores this species is not a powerful swimmer and captures zooplankton by using body current to draw them into its feeding apparatus (Main, 1928). From observations of gut contents, this species is reported to feed on mollusk larvae (Nelson, 1925), polychaete larvae (Main, 1928) and copepods (Nelson, 1925; Main, 1928; Bishop, 1967). Although Nelson (1925) reports that the ctenophore *Pleurobrachia* feeds on crustacean zoeae, it is not widely observed for *M. leidyi*. This is further supported by Cronin, Daiber, and Hulbert

(1962) in a study which quantitatively measured seasonal variations in zooplankton abundance in the Delaware River Estuary. During the summer of 1953 great numbers of *M. leidyi* were present and low numbers of the copepod *Acartia tonia* occurred with much higher relative numbers of crab zoeae. Cronin *et al.* (1962) speculated that *M. leidyi* was selectively feeding on the copepods, while the zoeae escaped predation due to their spines. Since *M. leidyi* readily ingests *R. harrisii* larvae in the laboratory, it is possible that the zoeae are effective in avoiding this ctenophore species under natural conditions by means of the shadow response.

In considering the plausibility of the shadow response, the ctenophore must be in a position within the water column such that it will cast a shadow upon a larva. Considering horizontal distributions, *M. leidyi* are found within estuarine-coastal water at salinities between 4–33‰ in the Chesapeake Bay (Bishop, 1972) with the largest distributions in the Beaufort, North Carolina area between about 2–23‰ (Schwartz and Chestnut, 1974). At 25° C *R. harrisii* larvae develop at salinities between 5 and 40‰ with the greatest survival at salinities 15 to 25‰ (Costlow, Bookhout and Monroe, 1966). In the Newport River Estuary, North Carolina, stage I zoeae are found between about 4 and 30‰ (Pinschmidt, 1963).

Considering vertical distribution, during the day *M. leidyi* is observed at the surface when it is calm and at shallow depths under windy conditions (Nelson, 1925). Based upon the phototactic responses by light- and dark-adapted larvae, Forward (1974a) predicted a diurnal vertical migration pattern of an ascent during the day and descent at night. This pattern is partially supported by Pinschmidt (1963) who collected stage I zoea in surface plankton tows during the day. Some larvae were also observed in bottom samples, but the overall vertical distributions were not measured throughout the day. The lighting condition necessary for the shadow response is that the initial light intensity be sufficient to induce a positive phototaxis in the larvae. This is consistent, since a positive phototaxis would contribute to the predicted vertical ascent by zoeae during the day. Therefore both specimens of *M. leidyi* and stage I zoeae are predicted to occur in similar salinity areas within estuarine areas and near the surface during the day.

The threshold for the shadow response occurs at an intensity equivalent to about a 0.5 O.D. decrease. Considering the apparent O.D./cm at 500 nm of the small size ctenophore (Fig. 5), animals larger than about 2.2 cm in length and 1.6 cm in width are predicted to have a longitudinal and transverse O.D. greater than 0.5. According to Main (1928) the adult feeding apparatus occurs in animals larger than 0.8 cm in length. This then indicates that there exists an animal size range which would not attenuate the light sufficiently to initiate the sinking response. Nevertheless, medium and large size adults are larger than this size and could cause this response (Fig. 5).

Thus, the shadow response by crustacean stage I zoeae consists of a passive sinking response which is followed by a negative phototaxis and active swimming, if the light intensity is lowered to a particular absolute level. This behavior is appropriate for avoiding zooplankton predators like ctenophores which do not actively pursue their prey but rather catch those organisms that are swept into their feeding apparatus. In addition, since ctenophores such as *M. leidyi* occur near the surface and thereby cast a downward shadow, sinking is a type of behavior that would always move a zoea away in a downward direction unless this was prevented by



vertical current flows. Light intensity should continually decrease as the predator is approached. Thus, the sinking response is possibly the initial avoidance behavior which occurs some distance away from the predator, while the negative phototaxis occurs at a closer distance. The negative response would be more effective for avoidance since it involves rapid swimming directed away from the light intensity decrease. These behaviors could be used to avoid other predators, *i.e.*, coelenterate medusae such as *Chrysaora quinquecirrha* and other ctenophore species. In addition, they probably are not limited to just stage I zoeae, as they are subjectively observed in all other zoeal stages.

#### NOTE ADDED IN PRESS

A recent publication on the distribution and feeding preferences of *Mnemiopsis leidyi* (Burrell and van Engel, 1976) presents evidence in support of the shadow response in *Rhithropanopeus harrisi*. They report that brachyuran larvae were rarely observed in the digestive system of the ctenophore and that "zoeae of the xanthid crab *Rhithropanopeus harrisi* were present in large numbers coincident with *M. leidyi* in the summer of 1966, but were apparently not preyed on, even though they were among the smallest (0.7 mm mean length) planktonic animals." Although the authors suggest that spines may deter predation, an alternate explanation is that the zoeae avoid the ctenophores by means of the shadow response described above.

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#### SUMMARY

1. A shadow response consisting of oriented movement to light and gravity was studied by means of a closed circuit television system for stage I zoeae from the crab *Rhithropanopeus harrisi*.

2. If larvae are irradiated at an intensity that induces positive phototaxis and the light is extinguished, both light- and dark-adapted larvae show a descent. Since this response involves passive sinking, it is termed a sinking response. The minimum time that the light must be extinguished to evoke the response is 20 to 30 milliseconds.

3. If the light intensity is reduced to a level that should induce negative phototaxis, light-adapted larvae show a sinking response followed by a negative phototaxis. The time delay between the responses is related to the initial stimulus intensity and duration.

4. The minimum decrease in intensity that induces the sinking response is equivalent to a reduction by a 0.5 O.D. neutral density filter while the maximum response occurs at optical densities of 1.0 to 1.1 and greater. These values are independent of stimulus time, intensity, and direction.

5. It is argued that these behaviors are appropriate for avoiding zooplankton predators like ctenophores which do not visually sight and actively pursue their



prey. The ctenophore *Mnemiopsis leidyi* is abundant in the Beaufort, North Carolina, area and spectrophotometric determinations of this species' apparent O.D. indicate that animals larger than a certain size attenuate the light sufficiently to evoke the shadow response.

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